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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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**Published:**
— *with international search report*

(88) Date of publication of the international search report:
28 September 2006

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: OPTIMIZED EXPRESSION OF HPV 58 L1 IN YEAST

(57) Abstract: Synthetic DNA molecules encoding the HPV58 L1 protein are provided. Specifically, the present invention provides polynucleotides encoding HPV58 L1 protein, wherein said polynucleotides are codon-optimized for high level expression in a yeast cell. The synthetic molecules may be used to produce HPV58 virus-like particles (VLPs), and to produce vaccines and pharmaceutical compositions comprising the HPV58 VLPs. The vaccines of the present invention provide effective immunoprophylaxis against papillomavirus infection through neutralizing antibody and cell-mediated immunity and are also useful for treatment of existing HPV infections.

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/037372

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/37 C12N15/81 A61K39/12 A61K48/00 C07K14/025

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | TOBERY T W ET AL: "Effect of vaccine delivery system on the induction of HPV16L1-specific humoral and cell-mediated immune responses in immunized rhesus macaques" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 21, no. 13-14, 28 March 2003 (2003-03-28), pages 1539-1547, XP004412501 ISSN: 0264-410X the whole document | 1-29 |
| A | WO 01/14416 A (MERCK & CO., INC; NEEPER, MICHAEL, P; MCCLEMENTS, WILLIAM, L; JANSEN,) 1 March 2001 (2001-03-01) claims 1-7,24-30; sequence 1 | 1-29 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 10 May 2005 | 19/05/2005 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Schulz, R |

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Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | ZHOU JIAN ET AL: "Papillomavirus capsid protein expression level depends on the match between codon usage and tRNA availability" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 73, no. 6, June 1999 (1999-06), pages 4972-4982, XP002164427 ISSN: 0022-538X abstract the whole document | 1-29 |
| A | LIU W J ET AL: "Polynucleotide viral vaccines: codon optimisation and ubiquitin conjugation enhances prophylactic and therapeutic efficacy" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 20, no. 5-6, 12 December 2001 (2001-12-12), pages 862-869, XP004312531 ISSN: 0264-410X page 864 - page 868; figures 1-4 | 1-29 |
| A | SCHILLER J T ET AL: "PAPILLOMAVIRUS-LIKE PARTICLE VACCINES" NATIONAL CANCER INSTITUTE. MONOGRAPHS, US NATIONAL CANCER INSTITUTE, BETHESDA, MD, US, vol. 28, 2000, pages 50-54, XP008016223 ISSN: 0083-1921 the whole document | 1-29 |
| A | HOFMANN K J ET AL: "Sequence derterminination of human papillomavirus type 6a and assembly of virus like particles in Saccharomyces cerevisiae" VIROLOGY, ACADEMIC PRESS,ORLANDO, US, vol. 209, 1995, pages 506-518, XP002100680 ISSN: 0042-6822 page 506 - page 507 | 1-29 |
| A | JANSEN K U ET AL: "Vaccination with yeast-expressed cottontail rabbit papillomavirus (CRPV) virus-like particles protects rabbits from CRPV-induced papilloma formation" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 13, no. 16, November 1995 (1995-11), pages 1509-1514, XP004057408 ISSN: 0264-410X the whole document | 1-29 |

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/037372

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,A | WO 2004/084831 A (MERCK & CO. INC; JANSEN, KATHRIN, U; SCHULTZ, LOREN, D; NEEPER, MICHAE) 7 October 2004 (2004-10-07) abstract; claims 1-43 | 1-29 |

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/037372

## Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16 and 17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US2004/037372

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 0114416 A | 01-03-2001 | AT 284898 T | 15-01-2005 |
| | | AU 772611 B2 | 06-05-2004 |
| | | AU 7063900 A | 19-03-2001 |
| | | CA 2381991 A1 | 01-03-2001 |
| | | DE 60016765 D1 | 20-01-2005 |
| | | DK 1212358 T3 | 04-04-2005 |
| | | EP 1212358 A2 | 12-06-2002 |
| | | JP 2003511010 T | 25-03-2003 |
| | | WO 0114416 A2 | 01-03-2001 |
| | | US 2005075303 A1 | 07-04-2005 |
| WO 2004084831 A | 07-10-2004 | WO 2004084831 A2 | 07-10-2004 |

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

| | | |
|---|---|---|
| Applicant's or agent's file reference<br>PCT 21561 | **FOR FURTHER ACTION** | See item 4 below |
| International application No.<br>PCT/US2004/037372 | International filing date *(day/month/year)*<br>10 November 2004 (10.11.2004) | Priority date *(day/month/year)*<br>12 November 2003 (12.11.2003) |
| International Patent Classification (8th edition unless older edition indicated)<br>See relevant information in Form PCT/ISA/237 | | |
| Applicant<br>MERCK & CO., INC. | | |

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| ☒ | Box No. I | Basis of the report |
| ☐ | Box No. II | Priority |
| ☒ | Box No. III | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| ☐ | Box No. IV | Lack of unity of invention |
| ☒ | Box No. V | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| ☐ | Box No. VI | Certain documents cited |
| ☐ | Box No. VII | Certain defects in the international application |
| ☒ | Box No. VIII | Certain observations on the international application |

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

| | |
|---|---|
| | Date of issuance of this report<br>03 October 2006 (03.10.2006) |
| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland<br>Facsimile No. +41 22 338 82 70 | Authorized officer<br>Ellen Moyse<br>e-mail: pt05@wipo.int |

Form PCT/IB/373 (January 2004)

# PATENT COOPERATION TREATY



From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

2615

## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) | |
| Applicant's or agent's file reference<br>see form PCT/ISA/220 | **FOR FURTHER ACTION**<br>See paragraph 2 below |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US2004/037372 | 10.11.2004 | 12.11.2003 |

International Patent Classification (IPC) or both national classification and IPC
C12N15/37, C12N15/81, A61K39/12, A61K48/00, C07K14/025

Applicant
MERCK & CO., INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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Authorized Officer

Schulz, R

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**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2004/037372

**Box No. I Basis of the opinion**

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☒ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

**Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 16, 17 with regard to industrial applicability

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 16, 17 with regard to industrial applicability

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

| | | |
|---|---|---|
| the written form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |
| the computer readable form | ☐ | has not been furnished |
| | ☐ | does not comply with the standard |

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

**Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Yes: Claims | 1-29 |
| | No: Claims | |
| Inventive step (IS) | Yes: Claims | |
| | No: Claims | 1-29 |
| Industrial applicability (IA) | Yes: Claims | |
| | No: Claims | 16, 17 |

2. Citations and explanations

**see separate sheet**

**Box No. VIII Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

III.1 Claims 16 and 17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(I) PCT).

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

V.1 State of the art

V.1.1 Reference is made to the following documents:

D1: Tobery, T. W. et al. (2003) Effect of vaccine delivery system on the induction of HPV16L1 specific humoral and cell-mediated immune responses in immunized rhesus macacaques. Vaccine 21, no. 13 - 14, p. 1539 - 1546.

D2: WO 01/14416 A (Merck & Co., INC.)

D3: Zhou, J. et al. (1999) Papillomavirus capsid protein expression level depends on the match between codon usage and tRNA availability. J. Virol. 73 (6), 4972 - 4982.

D4: Schiller, J. T. and Lowy, D. R. (2000) Developing HPV virus-like particle vaccines to prevent cervical cancer: a progress report. J. Clinical Virol. 19, (1)-(2), 67 - 74.

D5: Hofmann, K. J. et al. (1995)Sequence determination of Human Papillomavirus Type 6a and assembly of Virus like Particles in a Saccharomyces cerevisiae. Virol. 209, 506 - 518.

D1 discloses the codon-optimised human papillomavirus (HPV) 16 L1 coding sequence having been expressed in yeast (*S. cerevisiae*) and used for the preparation of virus-like particle (VLPs). Their effectiveness as a vaccine delivery system was compared to other approaches, such as e.g. plasmid DNA and replication incompetent adenoviral vector. Moreover VLPs comprising more than the L1 protein, i.e. in addition a modified L2 as well as E1/E2/E7 have been disclosed (p. 1540, right-hand side column, last para).

D2 discloses synthetic DNA molecules encoding various HPV proteins (L1, E1, E2 and / or E7) from any serotype of HPV, but preferably one causing a pathological condition in humans. These synthetic DNA molecules can be modified in accordance to the invention, i.e. codon-optimised with regard to the codon usage of the preferred host cell. Moreover, these molecules are meant to be used as a polynucleotide vaccine and / or an immunogenic composition comprising ". . . a mixture of HPV type protein genes (for example, genes from HPV6, 11, 16 and 18), and / or it may also contain a mixture of protein genes (i.e. L1, E1, E2, and/or E/) (p. 6, l. 24 - p. 7, l. 7).

D3 describes a study showing that the efficiency of expression of three different genes (BPV L1, L2 and GFP) in dividing mammalian cells *in vitro* depends on their codon composition, i.e. it was found that both codon-optimised and unmodified PV late genes were transcribed in COS cells, but that only the codon-modified genes were translated. Codon-optimisation consisted of conservative replacement of the viral codons with those less frequently used in mammalian genes (p. 4972, last para - 4973, 1st para).

D4 reviews the state of the art with regard to the use of HPV VLPs to prevent cervical cancer, i.e. multivalent vaccines comprising VLPs from HPV type 16, 18, 31 and 45 (p. 72, left-hand side column, line 2 - 7).

D5 discloses the complete genome of HPV6a as well as heterologous expression of HPV6a L1 or L1 + L2 in *S. cerevisiae*. Self-assembly into virus-like particles (VLPs) was demonstrated for L1 as well as for L1 + L2 expressing strains. The alledged advantages of the yeast expression system are discussed (p. 507, left-hand side column, 1st para).

V.2 **Novelty** (Art. 33(1)(2) PCT)

V.2.1 The present application appears to be the first to disclose a codon-optimised nucleic acid sequence encoding the HPV58 L1 protein as well as related products such as vectors, host cells or virus like particles comprising it.

V.2.2 The subject-matter of claims 1 - 29 is considered as new over the state of the art in the sense of Art. 33(2) PCT.

V.3 **Inventive Step** (Art. 33(1)(3) PCT)

V.3.1 The present application does not meet the criteria of Art. 33(1) PCT, because the subject-matter of claims 1 - 29 does not involve an inventive step in the sense of Art. 33(3) PCT.

V.3.2 The document D1 is regarded as being the closest prior art to the subject-matter of claim 1 and discloses a codon-optimised nucleic acid molecule encoding HPV16 L1 being expressed in *S. cerevisiae* cells (p. 1540, left-hand side column, 3rd para, right-hand side column, last para).

V.3.3 The subject-matter of claim differs from this known codon-optimised nucleic acid molecule in that it encodes the L1 protein derived from HPV58.

V.3.4 The problem to be solved by the present invention may therefore be regarded as the provision of a codon-optimised nucleic acid molecule encoding the L1 protein of another HPV strain.

V.3.5 The solution proposed in claim 1 of the present application cannot be considered as involving an inventive step in the sense of Art. 33(3) PCT) for the following reasons:
Codon-optimisation is a method known and well-established in the art that has already been applied to several HPV genes of different strains (D1; D2; D3, table 1). The skilled person is thus sufficiently enabled to modify the coding sequences of the L1 gene of another HPV strain without having to exercise his / her inventive skill.
Moreover, D2 already suggested to modify the codons of the sequence of a

synthetic molecule of further HPV strains, e.g. HPV58 (p. 7, l. 1 - 4) according to those preferred by the projected host cell respectively (p. 6, l. 25 - 26). Advantages associated with the yeast expression system are known in the art (D5). It therefore appears straightforward to codon-optimise any sequence to be expressed in these cells in order to increase the efficiency of the procedure.

V.3.6 The same reasoning applies, mutatis mutandis, to independent claims 7, 10, 13 - 17 and 29 and consequently, said claims are also considered as not inventive.

V.3.7 Dependent claims 2 - 6, 8, 9, 11, 12 and 18 - 28 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step since D1 already discloses vectors, host cells, VLPs comprising the codon-optimised nucleic acids encoding HPV16 L1 or HPV 16L1 + E1/E2/E7 being prepared from *S. cerevisiae* cells (p. 1540, right-hand side column, last para). VLPs have moreover been used as a vaccine of rhesus macaques (D1: p. 1541, right-hand side column, last para) and multivalent VLP vaccines are considered as a straightforward approach in the state of the art (D4).

V.4 **Comment** (Art. 33(1)(3) PCT)

V.4.1 For the assessment of the present claims 16 and 17 the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**Re Item VIII**

**Certain observations on the international application** (clarity)

VIII.1 **Sufficiency** of **Disclosure** (Art. 5 PCT)

VIII.1.1 Although the description refers to virus-like particles (VLPs) comprised of recombinant L1 protein or recombinant L1 + L1 proteins of HPV58 (cf. p. 2, l. 28; p. 3, l. 30; p. 10, l. 33; p. 11, l. 17), subject-matter of claim 10 as well as of claims 11 - 29 referring back to it can not be considered as sufficiently disclosed in the sense of Art. 5 PCT and supported in the sense of Art. 6 PCT over the whole of their breadth since the VLPs disclosed (cf. Ex. 7, 8) all only comprise either wild type (58 L1) or "rebuilt" (58 L1 R) HPV L1 protein and not as well L2.

VIII.2 **Clarity** (Art. 6 PCT)

VIII.2.1 The application does not meet the requirements of Art. 6 PCT, because subject-matter of claim 1 does not clearly define the matter for which protection is sought:

VIII.2.1.1 The skilled person limited to the technical features provided in claim 1, i.e. the HPV45 L1 **amino acid** sequence of SEQ ID NO: 2, cannot be considered as sufficiently enabled to distinguish whether any nucleic acid sequence of the prior art encoding that known protein has been codon-optimised or not and in case it were, for what kind of host cell.

VIII.2.1.2 Moreover, it is known in the art that nucleic acids encoding HPV L1 molecules that have been codon-optimised according to the codon-usage in mammalian cells can be efficiently expressed in yeast cells (D1, D2). The term "codon-optimised for high level expression in a yeast cell" is thus considered as ambiguous and vague and does not define subject-matter of claim 1 as required by Art. 6 PCT.

VIII.2.2 The vague statement in the description (p. 13, l. 7 - 15) implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Art. 6 PCT) when used to interpret them.